

Designation	Species	Epitope	Western Blot	IHC	FACS	Epitope sequence
29C11	rabbit	Pro2	Yes	yes**	n.d.	IDELKEFLNQIDETLSNVE
31A5	rabbit	Pro3	Yes	yes**	yes	ELLQEFIDDNATTNAIDELK
6A1	rabbit	Pro2-3	Yes	n.d.	no	TTNAIDELKEFLNQ
14A12	rabbit	Pro3	Yes	n.d.	yes	ELLQEFIDDNATTNAIDELK
6B12	rabbit	Pro3	Yes	n.d.	yes	ELLQEFIDDNATTNAIDELK
2D3	rabbit	Pro5	Yes	n.d.	yes	SQHCYAGSGCPILLENVISKTI
16D8	rabbit	Pro3	Yes	n.d.	yes	ELLQEFIDDNATTNAIDELK
31-1H7	mouse	n.d.	Yes	n.d.	yes	
197-1H11	mouse	Pro5	Yes	n.d.	no	SQHCYAGSGCPILLENVISKTI
32-1G11	mouse	n.d.	Yes	n.d.	yes	
304-1A5	mouse	n.d.	Yes	n.d.	yes	
98-1F4	mouse	n.d.	Yes	n.d.	no	

Fig. 1A

pc.h.mam.6a1.cell-57.579.1.t7

CACCATGGAGACAGGCCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCA
GTCGCTGGAGGAGTCCGGCGGTGCGCTGGTAACGCCTGGAGGATCCCTGACACTCACCTGCAC
AGTCTCTGGAATCGACCTCAGTAGCTATGGAGTGGGCTGGGTCCGCCAGGCTCCAGGGAAGG
GGCTGGAATACATCGGAATCATTAGTAAATTGATAACACATACTACGCGAACTGGGCGAAA
GGCCGATTACCATCTCCAAAACCTCGTCGACCACGGTGGATCTGAAAATGACCAGTCTGACA
ACCGAGGACACGGCCACCTATTCTGTACCAGAGGGTCTTTTGATCCCTGGGGCCAGGCACC
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pc.h.mam.16d8.cell-22.394.1.t7

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AGTCTCTGGATTCTCCCTCAGCAGCTACGACATGACCTGGGTCCGCCAGGCTCCAGGGAAGGG
GCTGGAATGGATCGGAACCATAGTACTATTGGTAGCCATTTTACGCGAGCTGGGCGAGAGG
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pc.h.mam.16d8.cell-21.393.2.t7

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CCGATTACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATACCAATCCGACAACCGA
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AGTCTCTGGAATCGACCTCAGCACCTACGACATGACCTGGGTCCGCCAGGCTCCAGGGAAGG
GACTGGAATGGATCGGAACCATAGTACTTTGGTACCCCTTTTCCGCCAATTGGGCGAGAG
GCCGATTACCATCTCCAAGACCTCGACCACGGTGGATCTGAAAATCGCCAGTCCGACGACCG
AAGACACTGCCACATATTTTGTGGCAGATTGCGGATTGCTCATGATGGTGCCTTCTGGGGCC
CAGGCACGCTGGTCACCGTCTCCTCAGGGCAACCTAA

Fig. 1B

pc.h.mam.2d3.cell-65.576.1.f7

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CACAGTGTCTGGAATCGACCTCAATATCGATGCAATGAGCTGGGTCGCCAGGCTCCAGGGA
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CACGGCCACCTATTTTTGTGTGAGAGGGGTAGTTTTANTTTTGCTACCGCCTTGTTGGGGCCA
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ATNGGAACCATAGTANTTTGTGGTAATGGATAATACGCGACCTGGGCGAAAGGCCGATTAC
CATTTCCAAAACCTTGACCACCGTGGATTTGAAAATCACCAGTCCGACAACCGAGGACACGG
CCAAGTATTTTGTGGCAGATTCGGATTGCTGGTGATGGTGCTTTTGGGGCCGGGACGCT
GGTCACCGTNTCTCAGGGCAACCTAA

Fig. 1C

Pro-1	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-2	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-3	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-4	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-5	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-7	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-8	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-9	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Glob-2	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
Pro-20	MKLLMWLMLAALSQHCYAGSGCP LL ENWISK T INPQVSKTEYKELLQEFID DN ATTNAIDELKECFLNQ TD ETLSNVEVFMQLIYDSSLC DLF
N-terminal recombinant	GS GM KETAAAKFERQHMDSPDLGTDDDDKAMASDPNS..... <u>HCVAGSGCPLLENWISK</u>
	Peptide with Enterokinase and Thrombin cleavage sites Mammaglobin sequence

Fig. 2

Reactivity of Mouse Monoclonal antibodies to Mammaglobin with peptides and recombinants											
Antibody	Pro2	Pro-3	Pro-4	Pro-5	Pro-6	Pro-7	Pro-8	Glob-2	amma+Tlnal	recon	TRX
31-1H7	0.065	0.059	0.059	0.061	0.06	0.066	0.07	0.063	2.788	0.074	0.116
32-1G11	0.056	0.055	0.054	0.054	0.055	0.057	0.055	0.055	2.75	0.057	0.07
197-1H11	0.055	0.054	0.053	1.139	0.054	0.055	0.055	0.055	2.502	2.596	0.064
304-1A5	0.054	0.054	0.053	0.053	0.054	0.053	0.053	0.054	2.7	0.056	0.064
98-1F4	0.068	0.055	0.053	0.055	0.059	0.064	0.11	0.112	2.819	0.118	0.121
967	0.055	0.057	0.056	0.056	0.055	0.62	0.056	0.637	1.566	0.069	0.159
Blank	0.056	0.055	0.053	0.055	0.052	0.053	0.053	0.053	0.056	0.052	0.06

Fig. 3A

Mammaglobin rabbit monoclonal 6B12

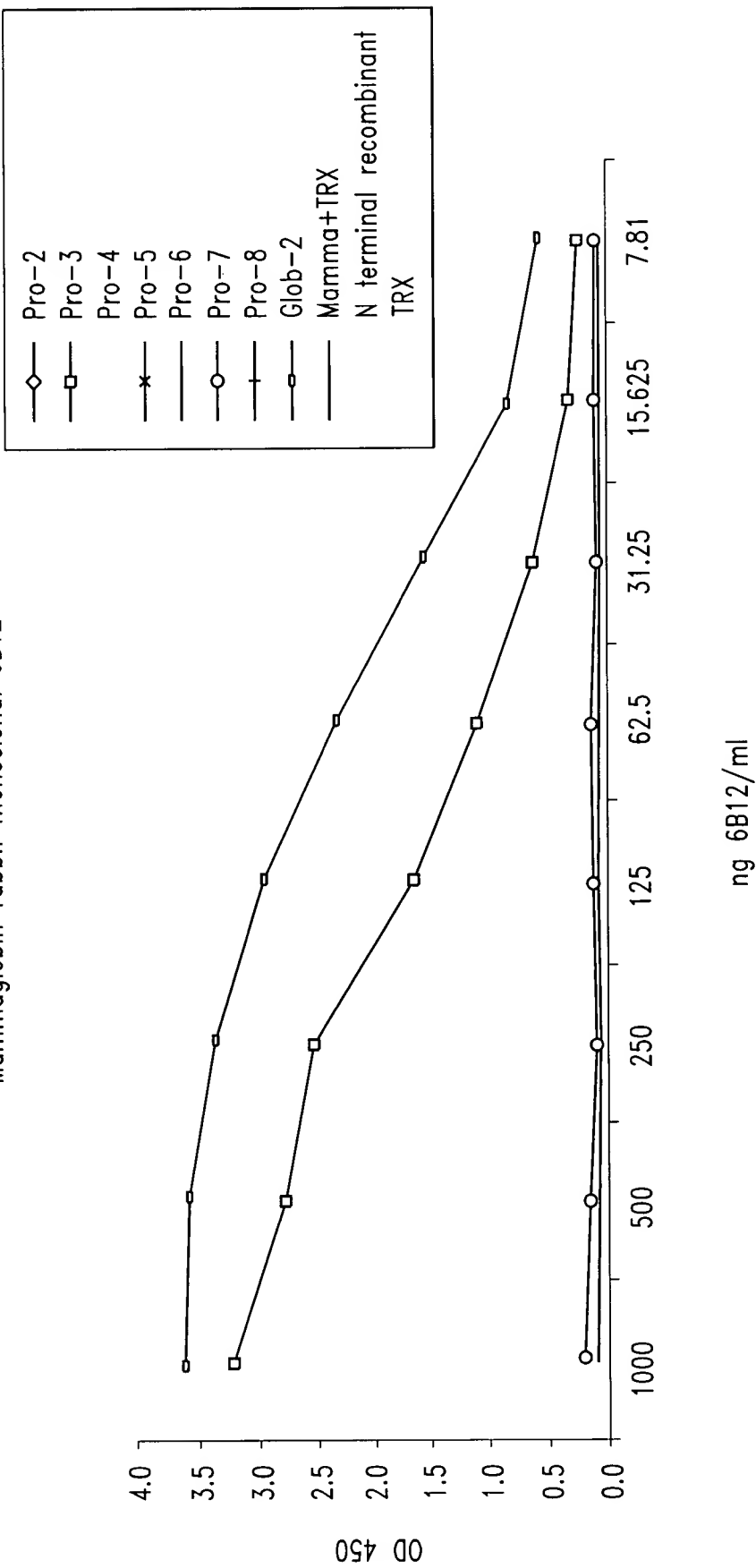


Fig. 3B

Mammaglobin rabbit monoclonal 29C11

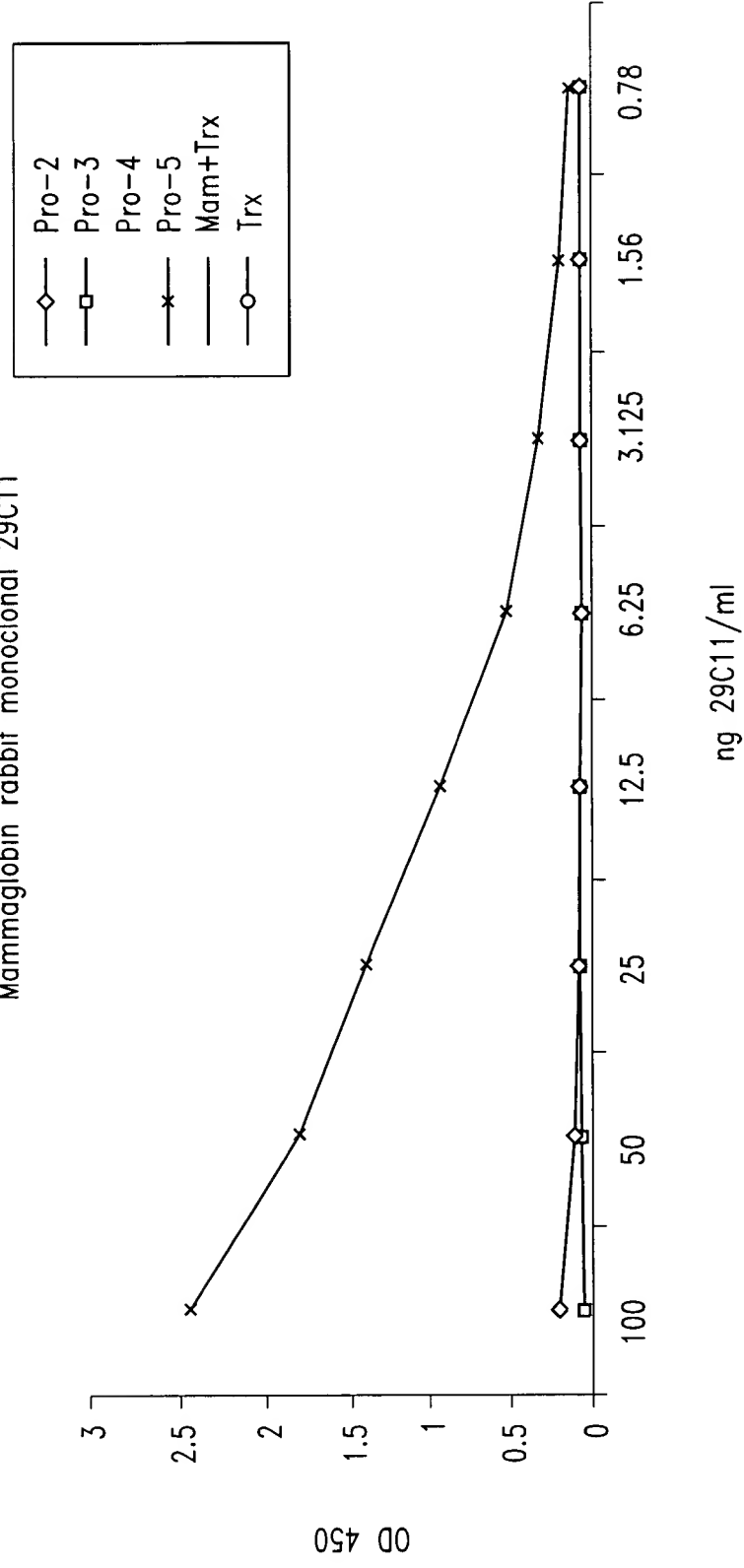


Fig. 3C

Mammaglobin rabbit monoclonal 2D3

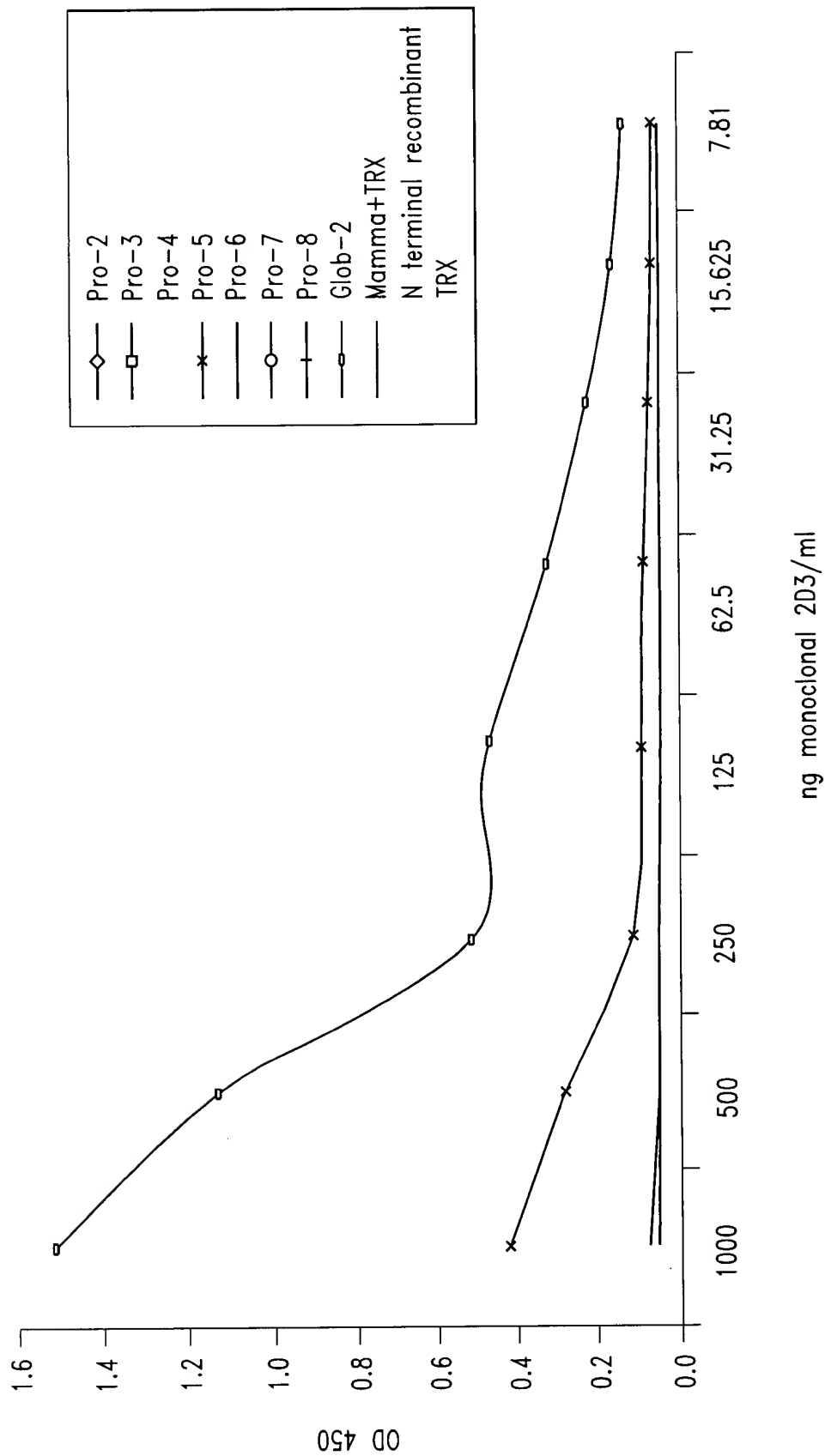


Fig. 3D

Staining of permeabilized human breast tumor cell line MDA-MB415 with rabbit anti-mammaglobin monoclonal antibodies

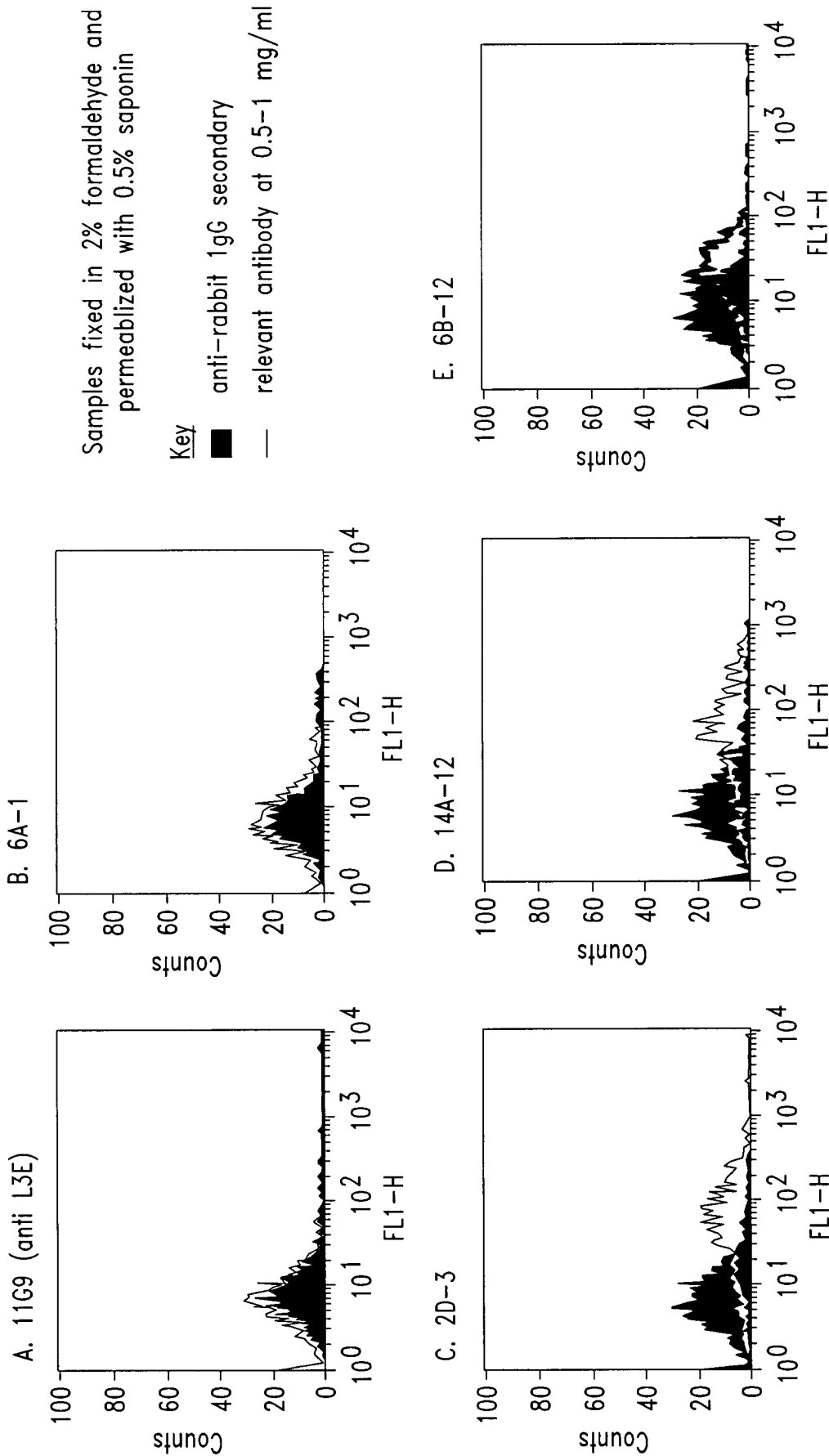


Fig. 4A

Staining of permeabilized human breast tumor cell lines
with murine anti-mammaglobin monoclonal antibodies

Key

- Secondary alone
- Primary at 1:10

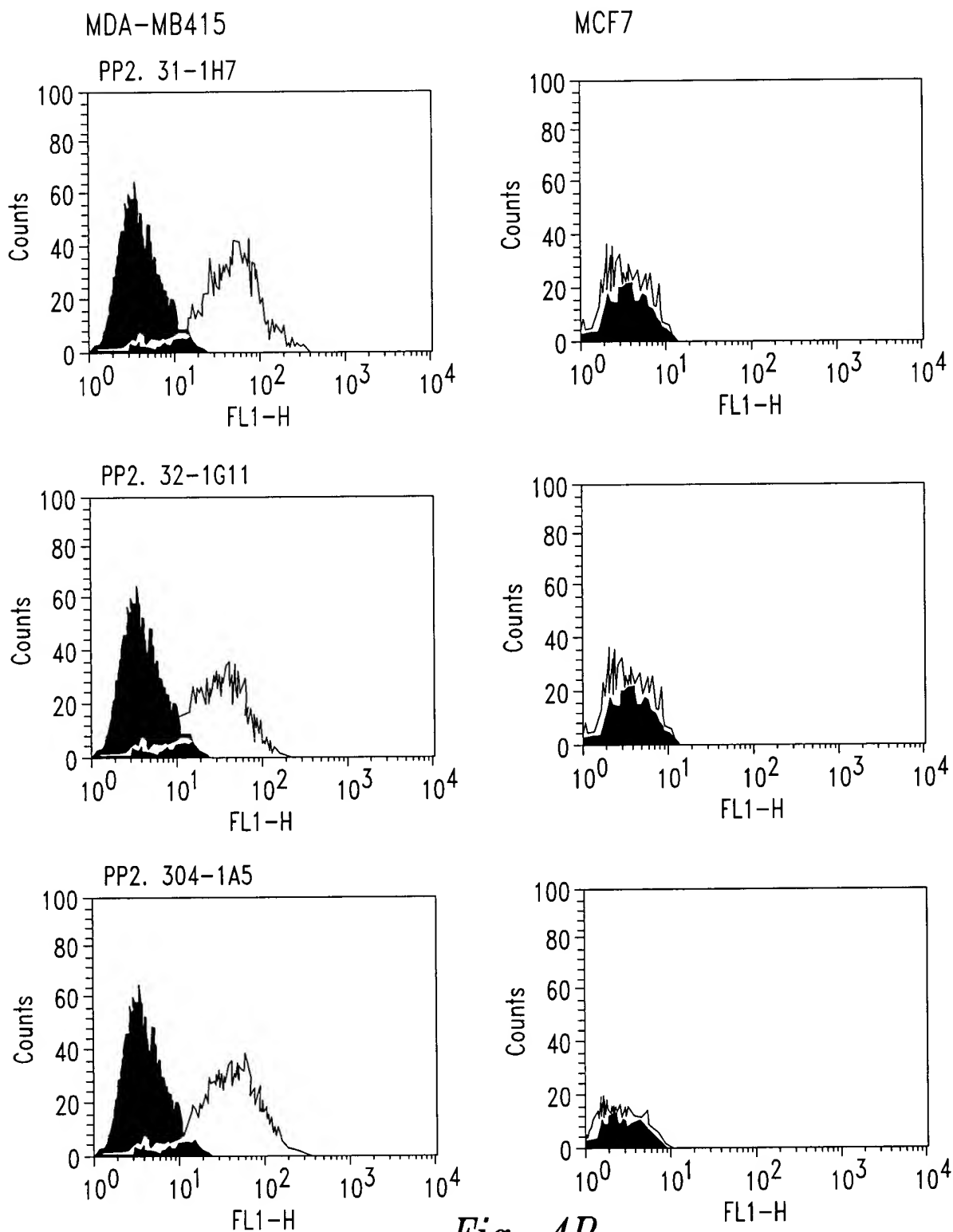
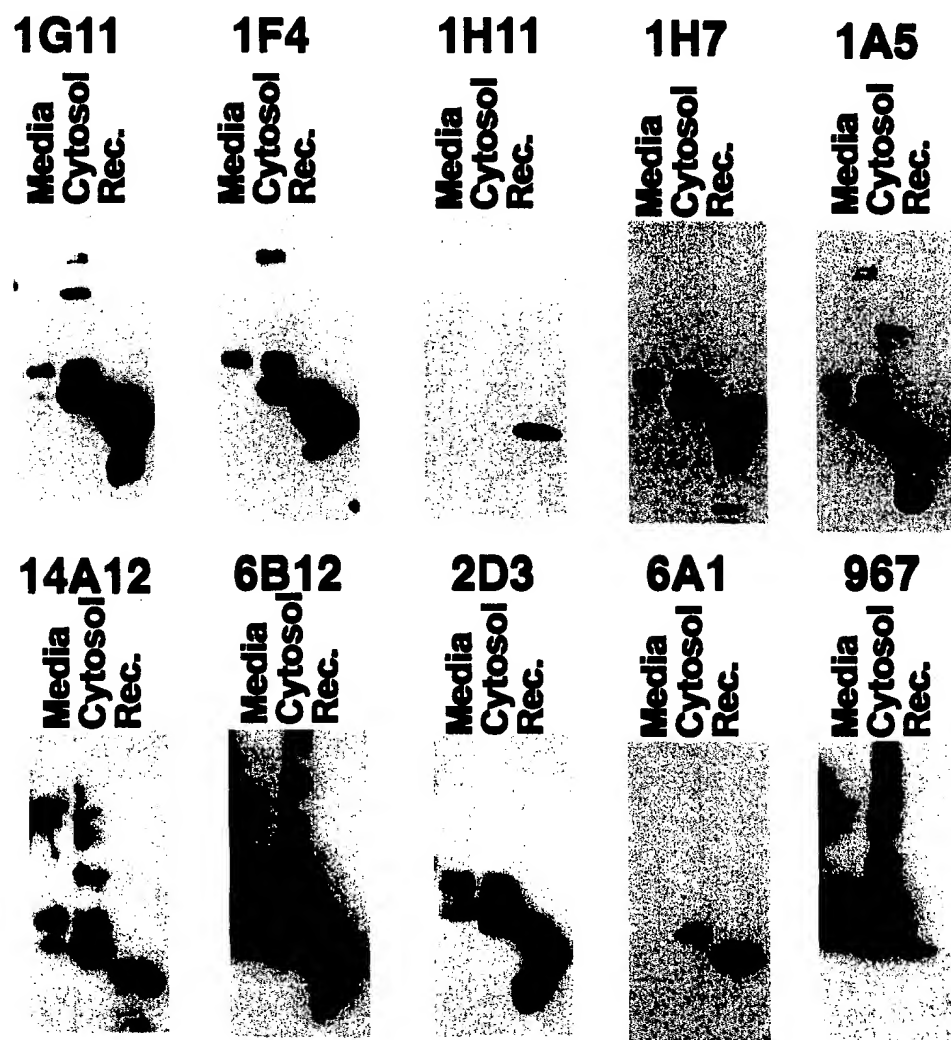


Fig. 4B

Western blot analysis of Mammaglobin from MB415 cells



Mouse monoclonal: 1G11, 1F4, 1H11, 1H7, 1A5
 Rabbit monoclonal: 14A12, 6B12, 2D3, 6A1
 Rabbit polyclonal: 967

Rec.: bacterially expressed recombinant mammaglobin

Fig. 5

403070 274250

IHC analysis of mammaglobin expression in normal tissue.

Normal Tissue	Mam-29C11/31A5
Breast	3-
Adrenal	0
Cervix	0
Colon	0
Duodenum	0
Gall bladder	0
Ileum	0
Kidney	0
Ovary	0
Pancreas	0
Paroud gland	0
Prostate	0
Skeletal muscle	0
Spleen	0
Testis	0

Fig. 6

4425260

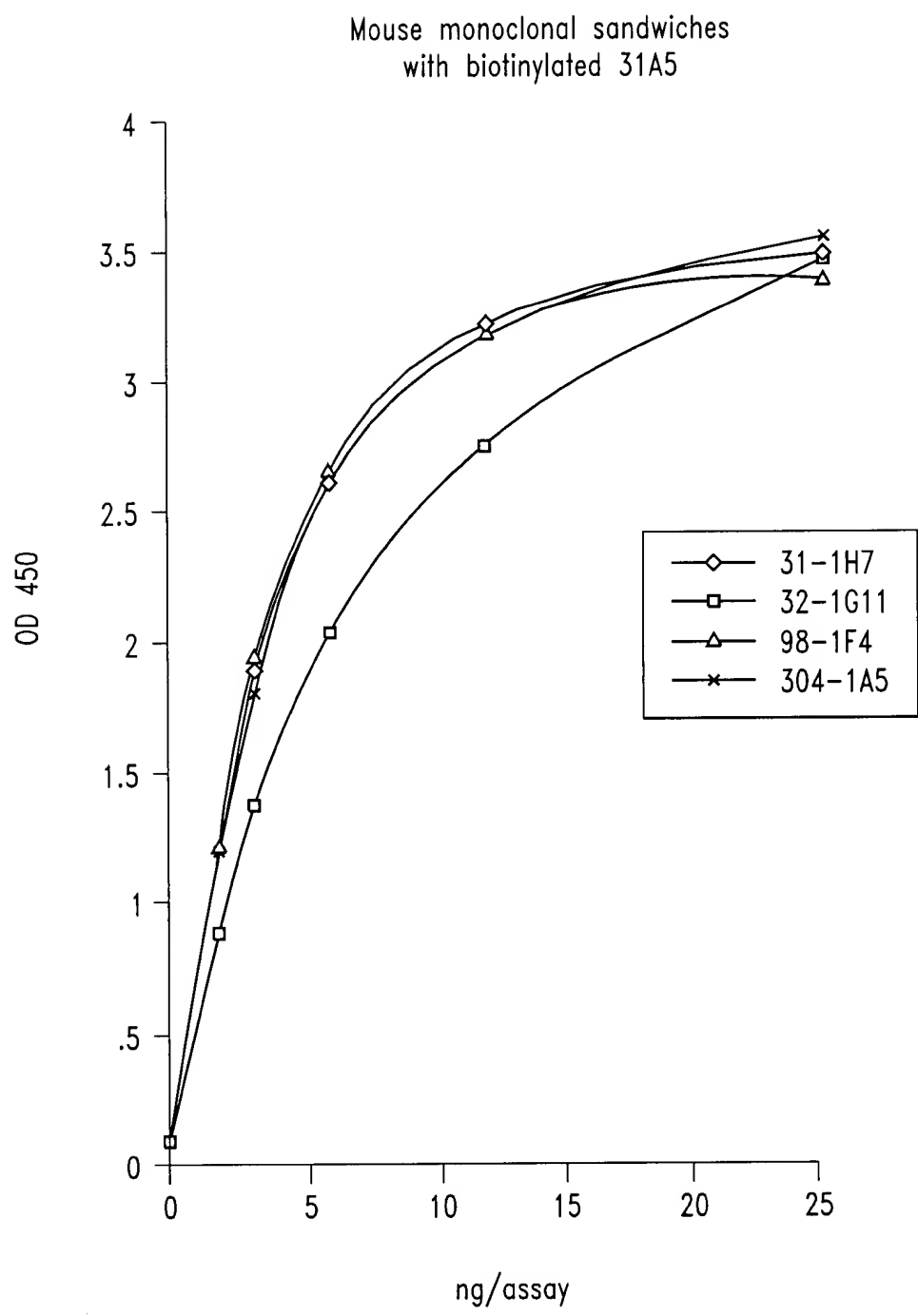


Fig. 7A

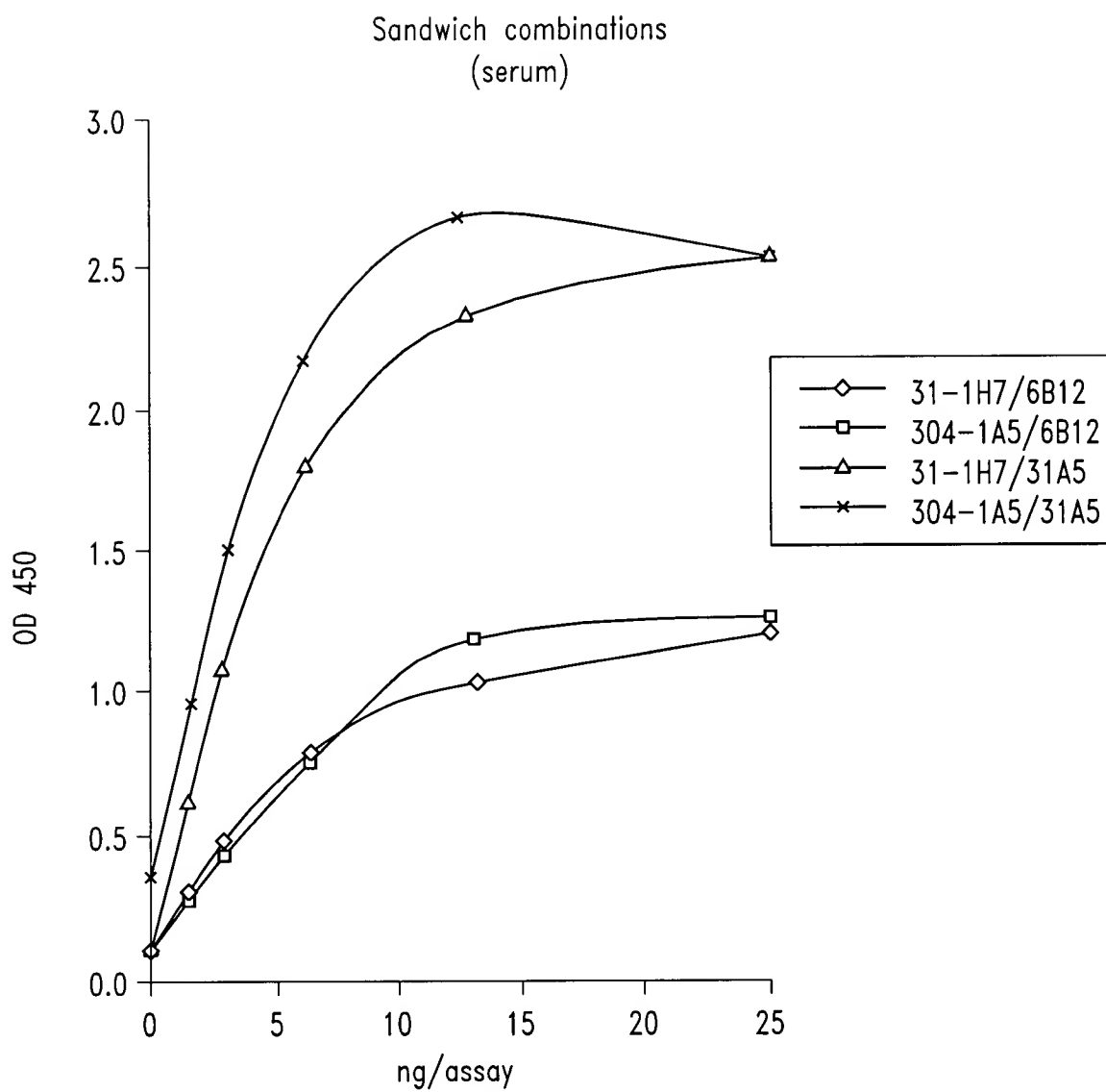


Fig. 7B

103070-24260

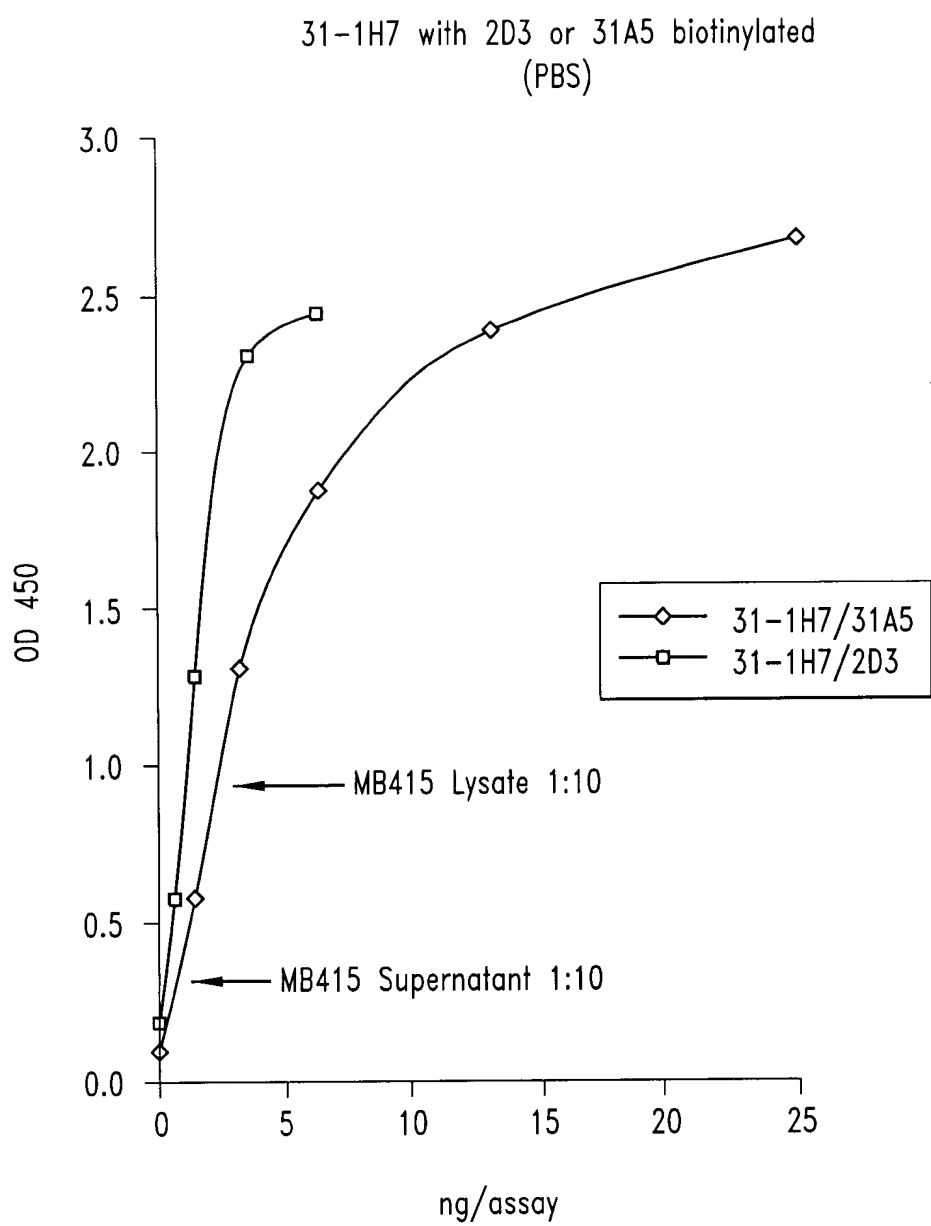


Fig. 7C

103070-24250

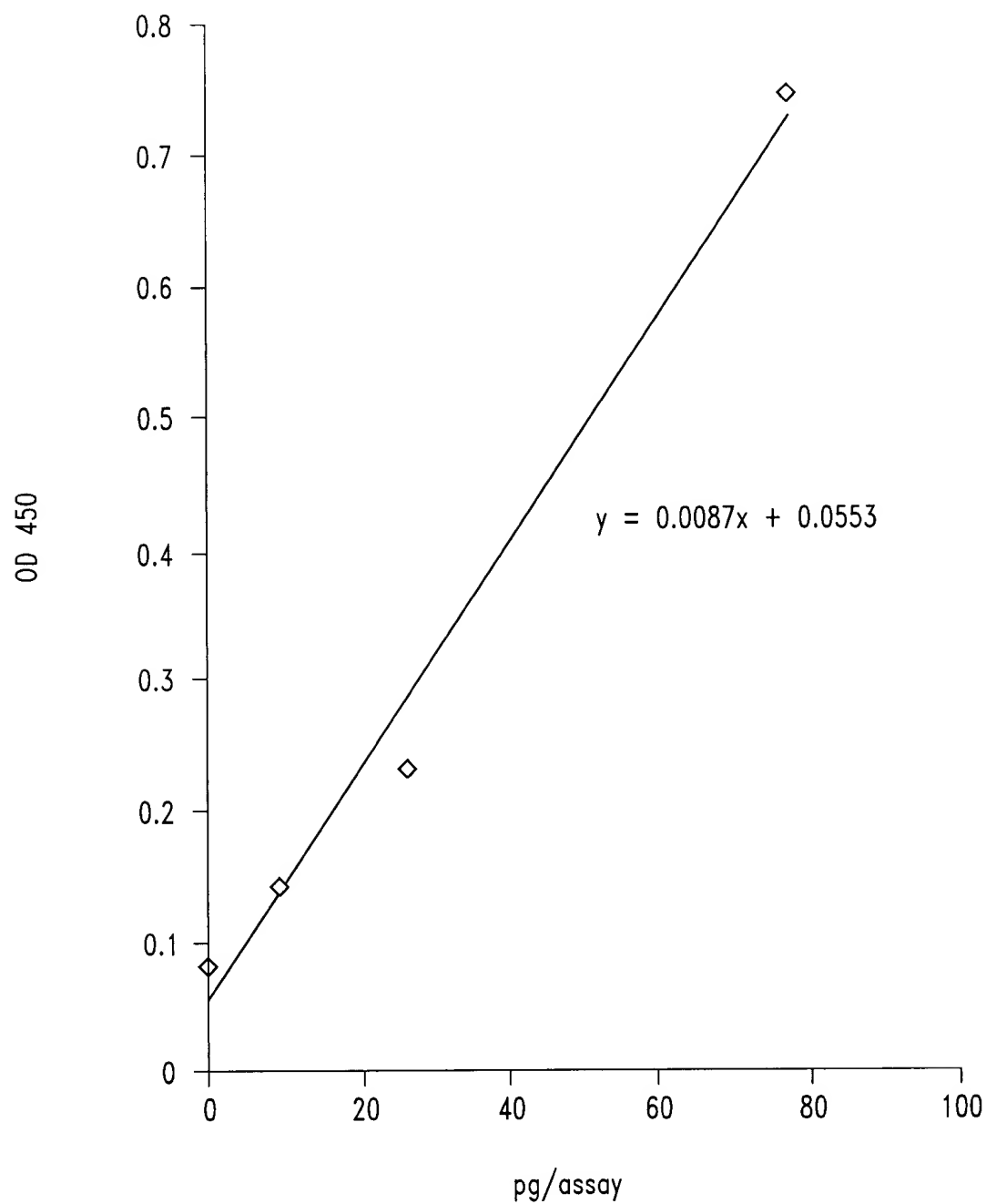


Fig. 8

Detection of mmamglobin in sera

Serum #	Status	Western	Mamaglobin [pg/ml]	OD	Mamaglobin [pg/ml]**	MRNA in blood*
6 (aka 3534)	BrCA	+	4980-9600	3.8	8732	not tested
3	BrCA	nd	560-1245	2.6	2392	+
4	BrCA	nd	311-622	1.7	1443	+
12	BrCA	nd	311-622	1.5	2298	weakly +
17	BrCA	nd	149-311	0.6	1498	+
11	BrCA	nd	149-311	0.6	847	+
10	BrCA	nd	74-149	0.38	356	nd
1	Normal F	nd	38-74	0.21	2333	not tested
18	Normal M	nd	38-74	0.2	636	not tested
8	BrCA	nd	38-74	0.19	284	nd
9	Normal F	nd	38-74	0.18	188	not tested
5	Normal F	nd	<33	0.16	43	not tested
2	Normal F	nd	<33	0.14	149	not tested
7	Normal F	nd	<33	0.13	96	not tested
14	Normal F	nd	<17	0.05	18	not tested
16	Normal F	nd	<17	0.01	363	not tested
13	Normal F	nd	<17	0.01	443	not tested
15	Normal F	nd	xxx	xxx	10.8	not tested

Fig. 9

1a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

2a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

3a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

4a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

5a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

6a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

7a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

8a MKLLMVLMLAALSQHCYAGSGCPLENNISK^TINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQ^TDETL^SSNVEVFMQLIYDSSLCDLF

peptide #	AA sequence	AA location within mmgb
1a	MKLLMVLMLAALSQHCYAGS	1-20
2a	ALSQHCYAGSGCPLENNIS	11-30
3a	GCPLLENNISK ^T INPQVSKT	21-40
4a	KTINPQVSKTEYKELLQEFI	31-50
5a	EYKELLQEFIDDNATTNAID	41-60
6a	DDNATTNAIDELKECFLNQ ^T	51-70
7a	ELKECFLNQ ^T DETL ^S SNVEVF	61-80
8a	DETL ^S SNVEVFMQLIYDSSLCDLF	71-93

Fig. 10

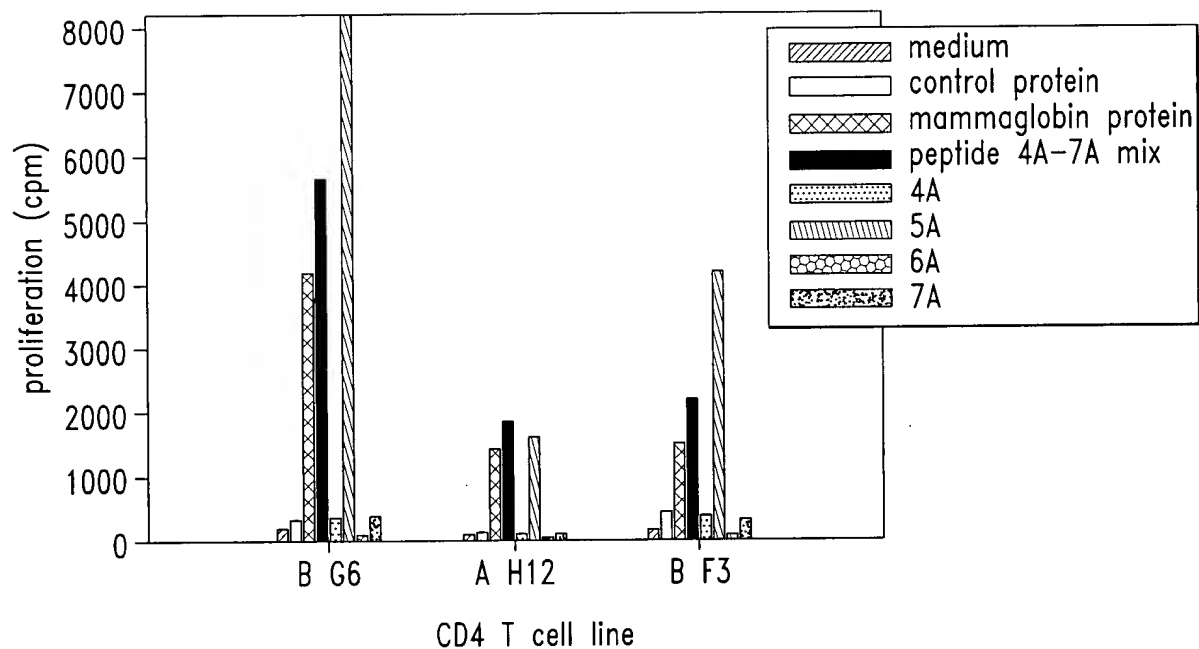


Fig. 11A

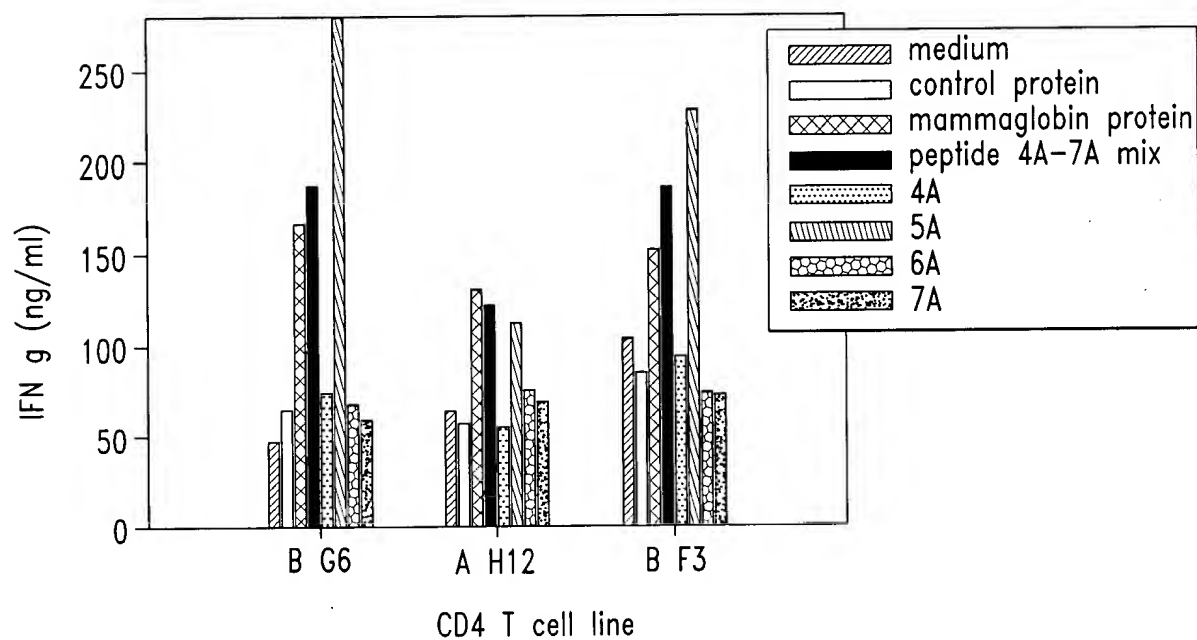


Fig. 11B

MKLLMVLMLAALSQHCYAGSGCPLENNISKVINPQVSKTEYKELLQEFIDDNATTNAIDELKECFLNQTDETLSNVEVFMQLIYDSSLCDLF

#	Start positon	sequence (length)	score
1	2	KLLMVLMLA (9)	148
2	3	LLMVLMLAA (9)	72
3	4	LMVLMLAAL (9)	60
4	66	FLNQTDETL (9)	48
6	83	LIYDSSLCDL (10)	151
7	2	KLLMVLMLAA (10)	148
8	80	FMQLIYDSSL (10)	71
9	58	AIDELKECFL (10)	26
10	45	LLQEFIDDNA (10)	17

Fig. 12

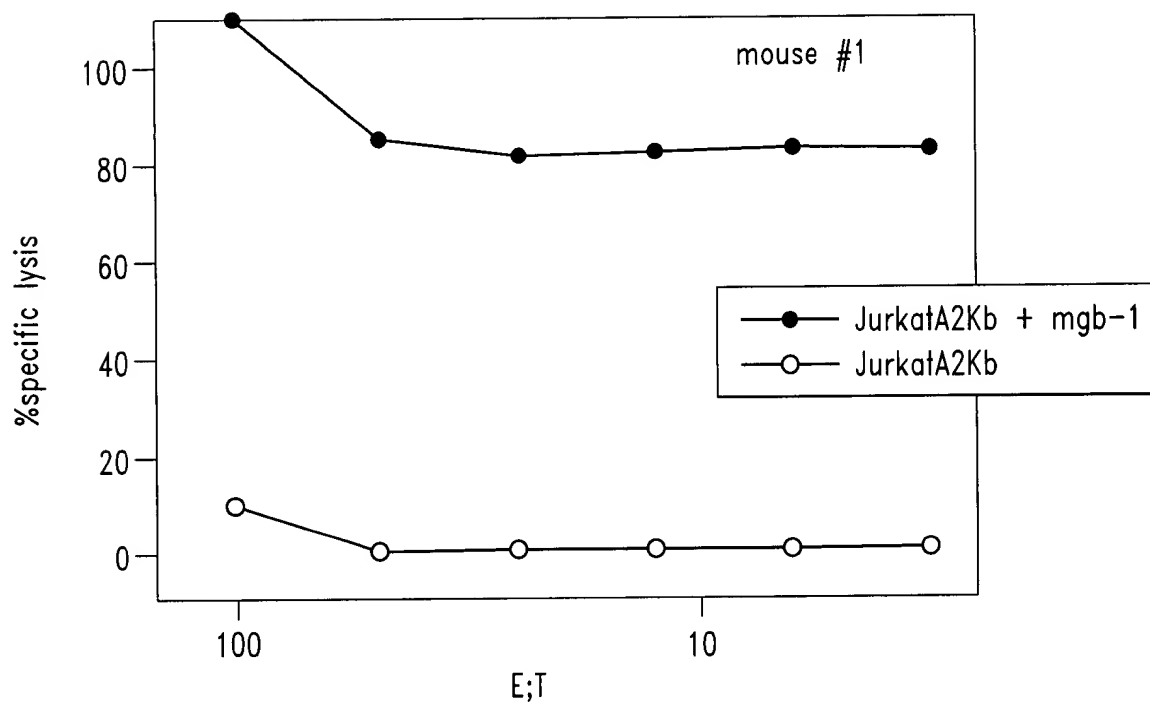


Fig. 13A

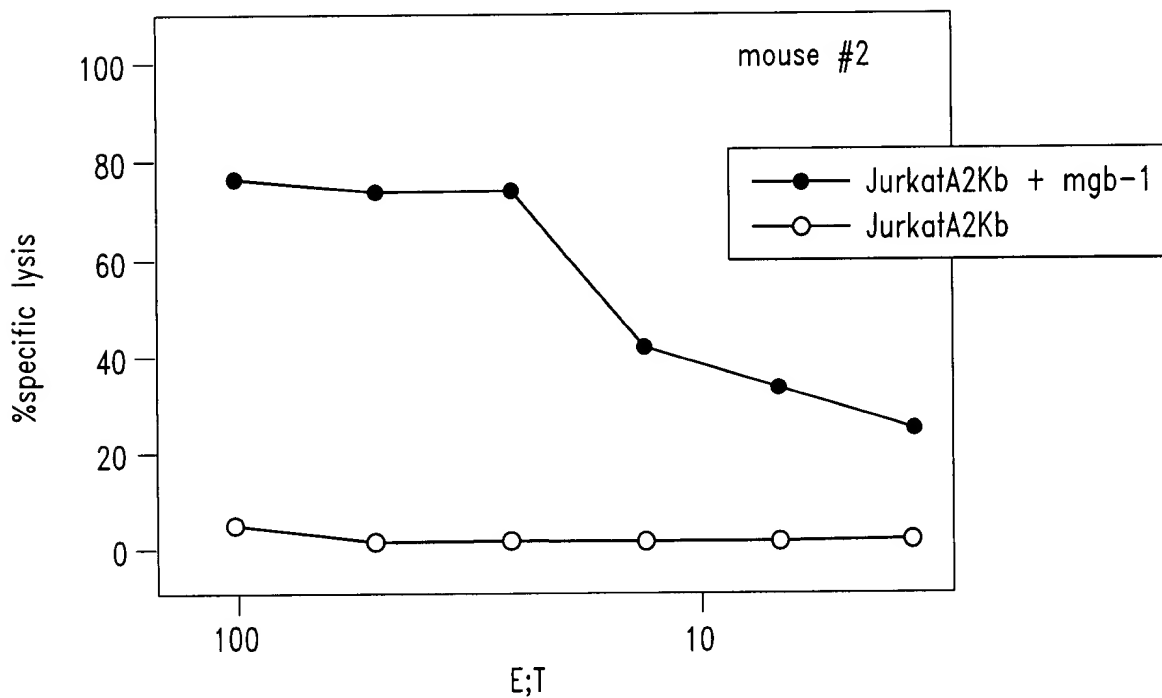


Fig. 13B

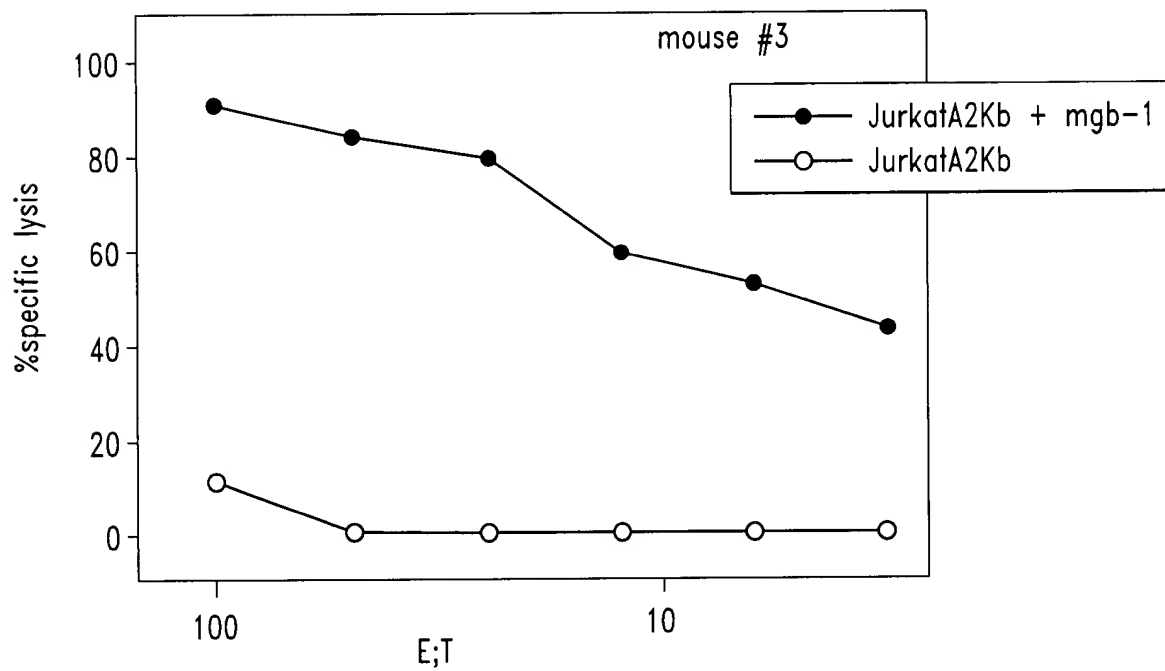


Fig. 13C

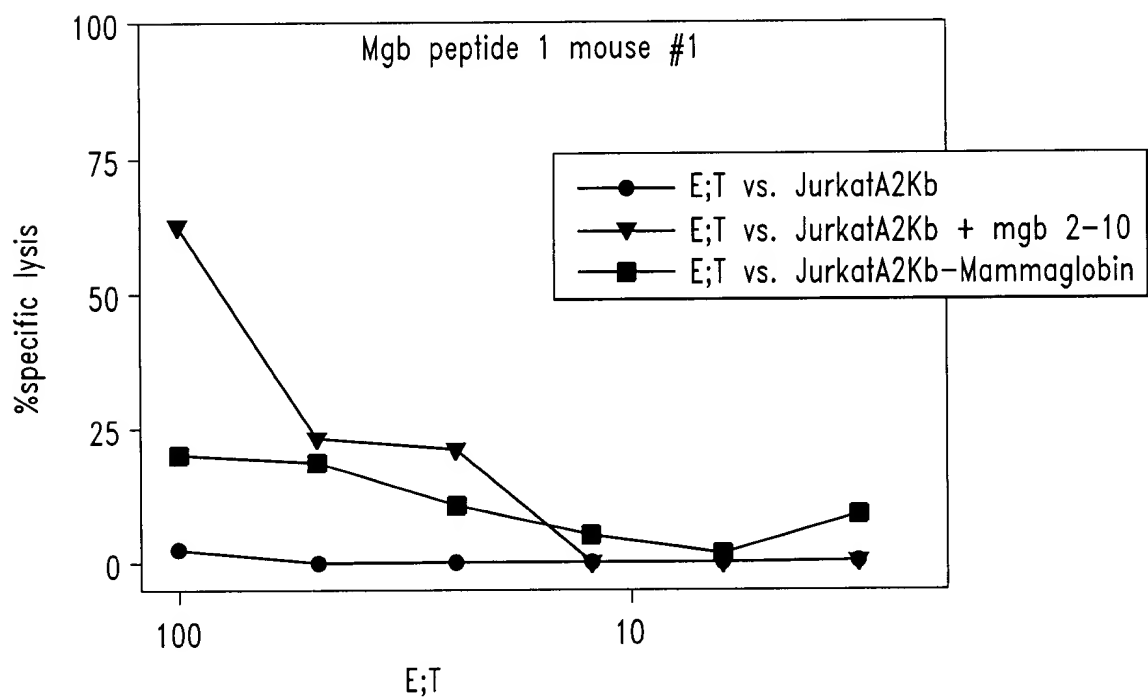


Fig. 14A

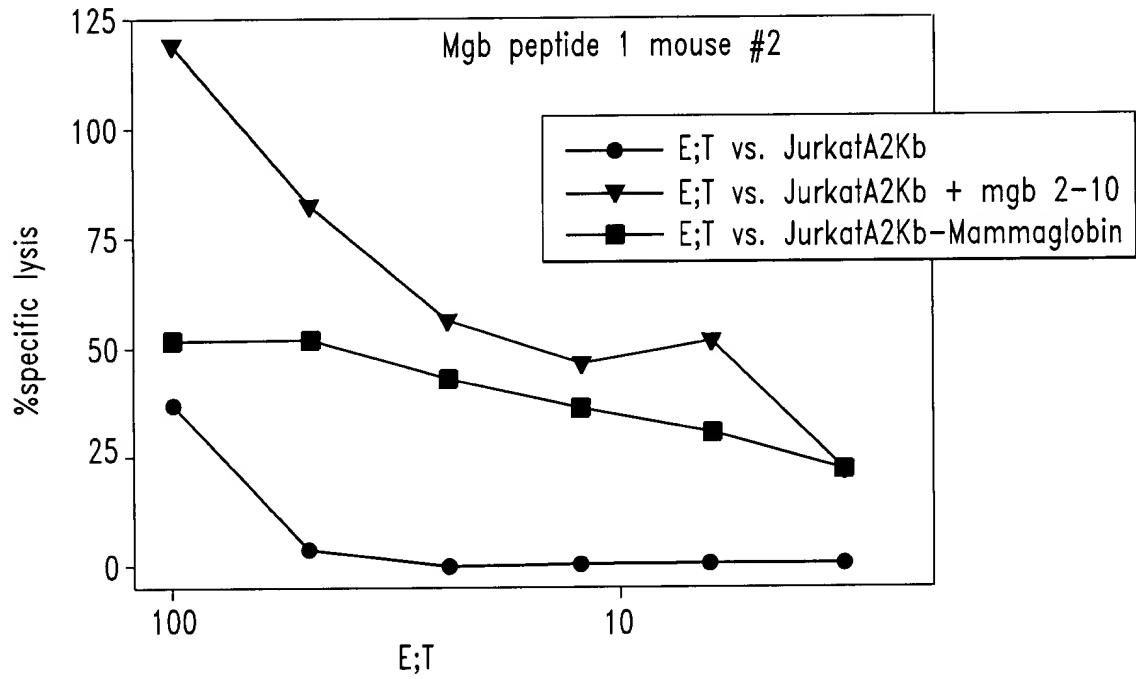


Fig. 14B

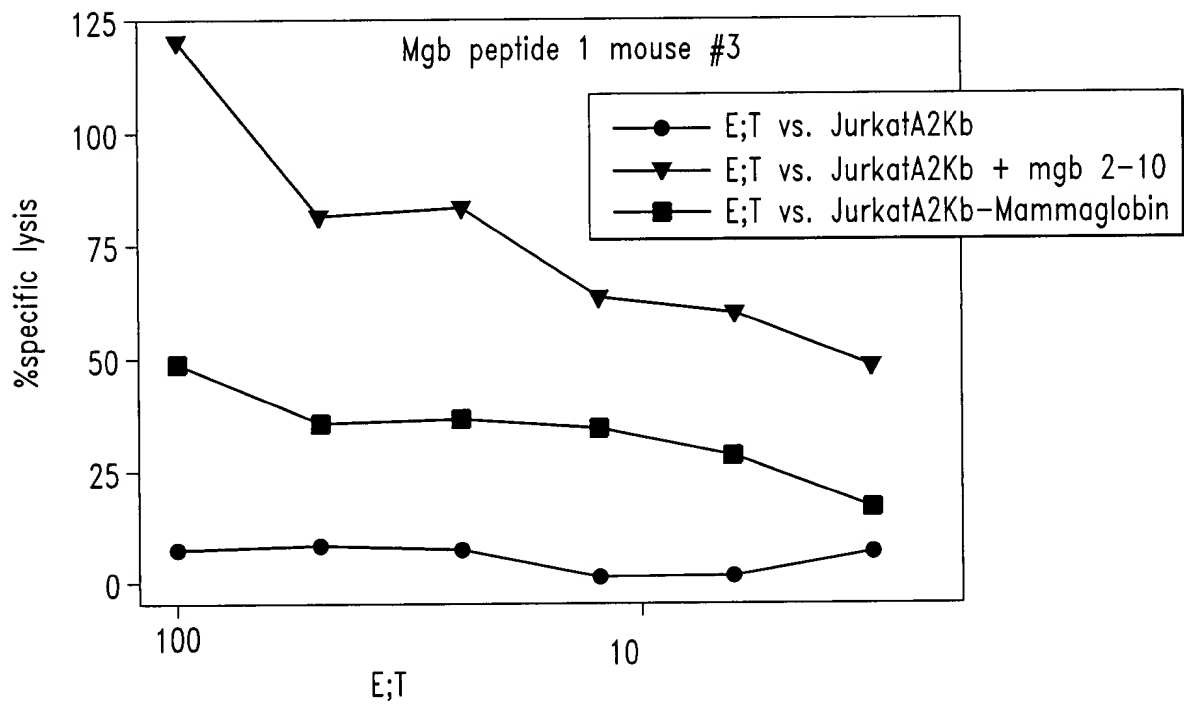


Fig. 14C

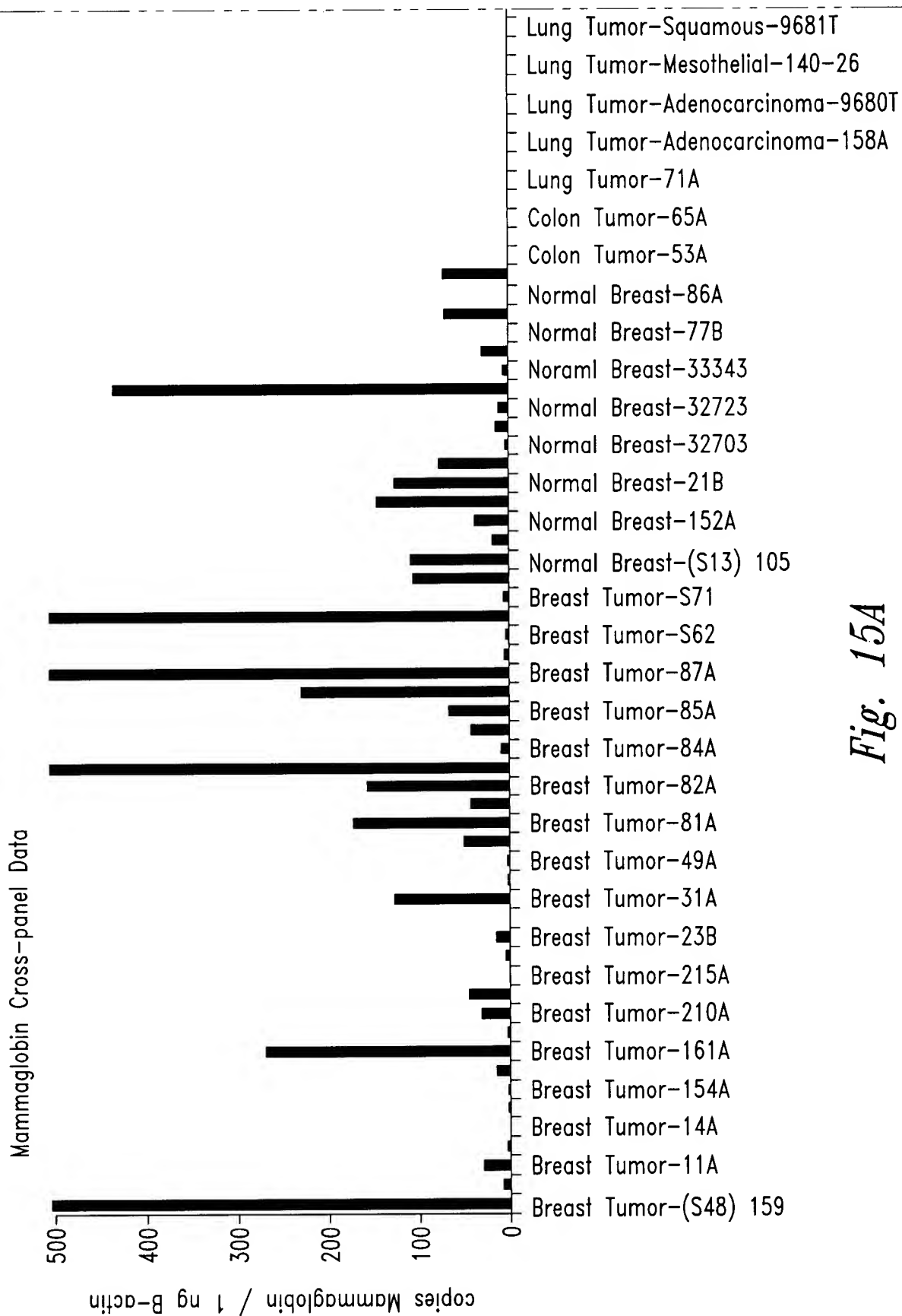


Fig. 15A

Normal Testes-4C
 Normal Stomach-73A
 Normal Stomach-137A
 Normal Stomach-137A
 Normal Small Intestine-66B
 Normal Skin-138A
 Normal Skin-60A
 Normal Skeletal Muscel-128A
 Normal Retina-32263
 Normal Ovary-93B
 Normal Lung-Clontech
 Normal Lung-58A
 Normal Lung-51C
 Normal Liver-56A
 Normal Liver-136A
 Normal Kidney-69A
 Normal Kidney-119A
 Normal Esophagus-1375
 Normal Colon-50B
 Normal Brain-Clontech
 Normal Brain-75A
 Normal Bone Marrow-74A
 Normal Bladder-S9-1
 Normal Aorta-1375
 Normal Prostate-131A
 Normal Prostate-48B
 Normal Prostate-45A
 Normal Prostate-34C
 Normal Prostate-117A
 Prostate Tumor-40A
 Prostate Tumor-35A
 Prostate Tumor-135A
 Prostate Tumor-115A
 Ovary Tumor-120A
 Lung Tumor-Squamous-96A

Fig. 15B

MB415 cells versus copy number for
Mammaglobin

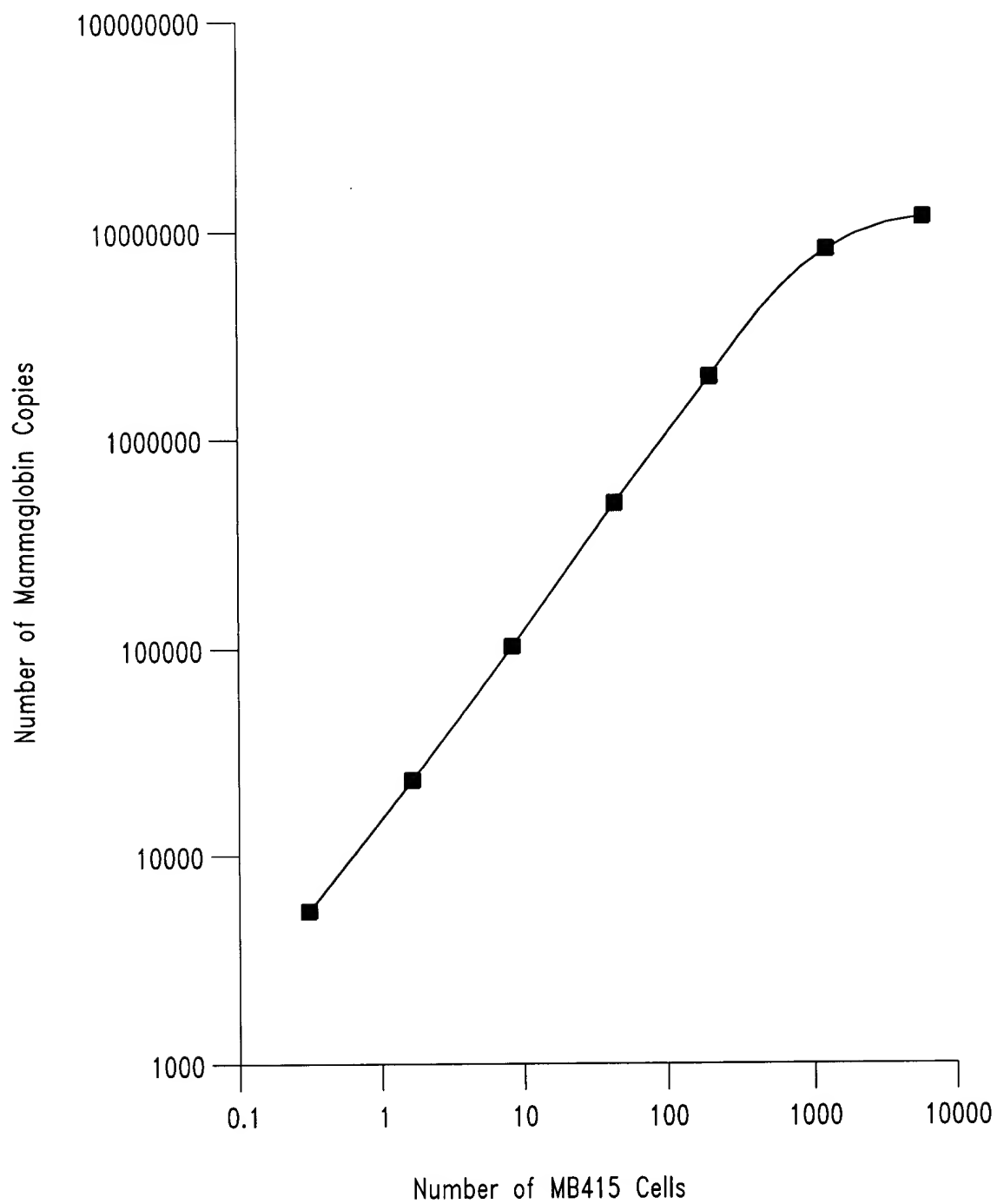


Fig. 16

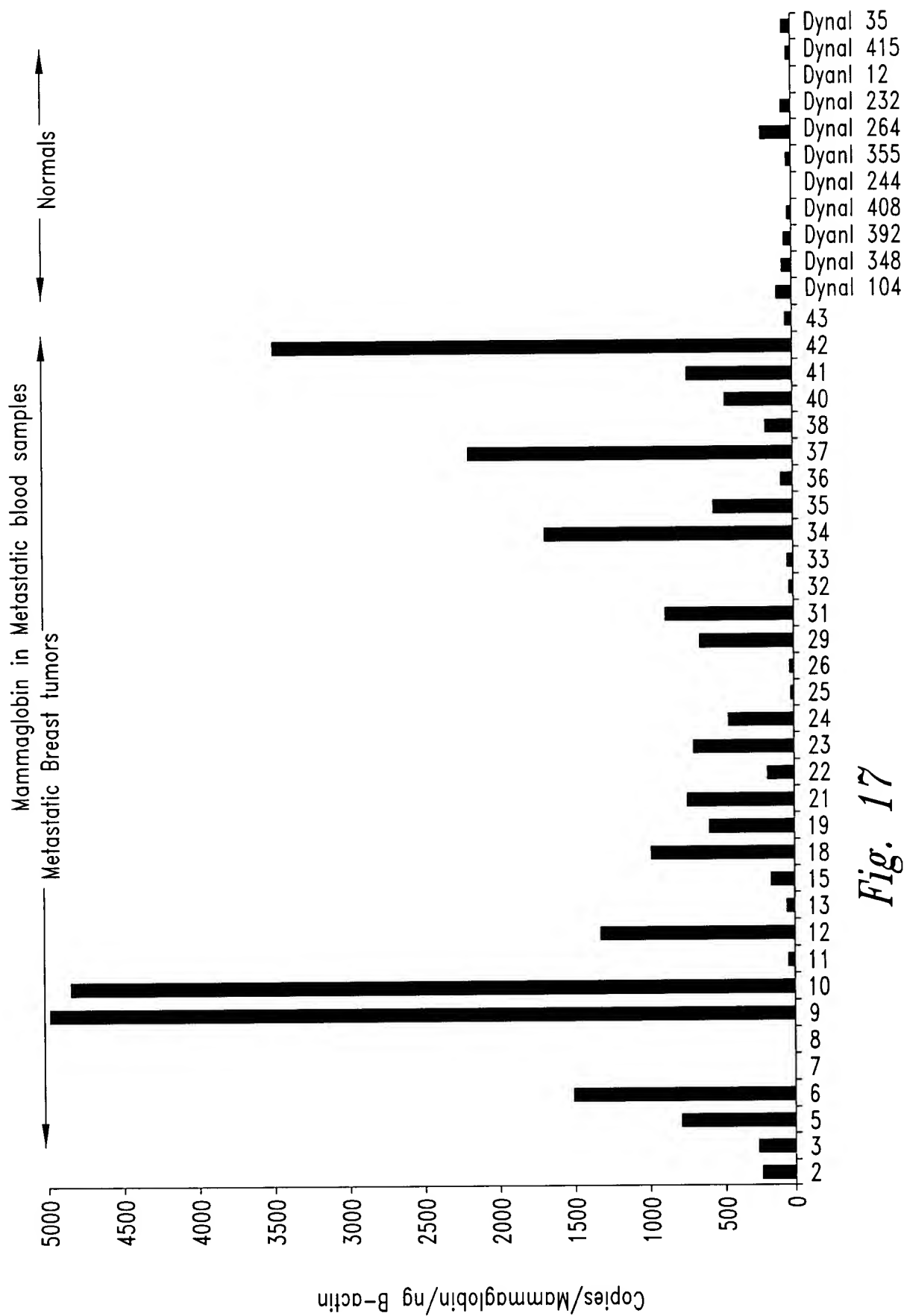


Fig. 17

FIGURE 18

D11.7 mpb CD4 proliferation - large assay #2												
line #	name	priming pct	media	DMSO	1A-7A	3A	5A	7A	mpb B 5A	Hmammm 10	Hmammm 1	Hmammm 1
1	AB:G8	5A	551	549	5478	454	12589	329	886	5939	1944	1222
2	AB:G11	5A	155	84	13737	159	17250	137	596	18027	14327	4598
3	AB:E7	5A	582	551	7815	198	12876	485	1284	11457	8696	1890
4	AB:H12	5A	1309	1725	18113	865	5850	1264	295	1158	4232	922
5	AB:A7	1A-7A	588	683	15648	4500	112	22045	417	2758	1822	792
6	AB:A9	1A-7A	478	378	6839	396	428	4095	135	418	282	136
7	AB:B8	1A-7A	1802	1802	29047	9277	2828	5838	1177	617	883	2307
8	AB:C9	1A-7A	2142	2258	18814	3158	2836	11835	2954	925	1008	780
9	AB:G7	1A-7A	1553	992	7754	2004	3355	3629	482	5405	3744	2524
10	AB:G9	1A-7A	1607	1577	7583	1489	3487	1752	689	5639	2388	2288
11	AB:H12	1A-7A	3101	2823	23406	24070	2354	8379	2353	15009	5888	2759
12	AB:H4	1A-7A	878	691	16789	574	3658	11797	478	1157	1189	667
13	CD:A4	1A-7A	124	520	20856	21542	805	3049	167	16009	4098	455
14	CD:A5	1A-7A	1439	328	12641	22252	2925	1358	563	4822	883	211
15	CD:C7	1A-7A	76	48	67	86	39	38	40	106	91	79
16	AB:G7	5A	173	477	1073	184	127	489	562	985	543	629
17	AB:H12	5A	948	329	2001	849	1301	266	380	775	1340	355
18	AB:C10	5A	223	181	486	254	341	97	204	340	252	133
19	AB:C11	5A	247	164	22728	148	15534	181	222	20054	21733	8331
20	AB:G8	5A	2125	2048	2408	1618	885	1498	1217	4175	2548	1845
21	AB:G7	5A	91	167	1688	162	2582	93	70	1013	518	178
22	AB:H2	5A	411	720	21053	271	11029	157	220	10420	9317	6009
23	AB:D1	5A	222	608	204	412	276	125	57	1991	1171	741
24	AB:E9	5A	315	457	390	191	1195	177	135	847	286	341
25	AB:G8	1A-7A	485	295	5014	70	2148	48	465	20516	12078	5873
26	AB:H4	1A-7A	545	192	14133	180	891	7518	105	2847	869	578
27	AB:D12	1A-7A	1852	1522	13318	8496	3131	4081	946	20077	11118	8972
28	AB:D1	1A-7A	1448	1614	4205	1199	1186	1822	430	5215	3124	3258
29	AB:H1	1A-7A	6572	3885	18528	14527	1817	13029	1587	11289	4834	1988
30	AB:A7	1A-7A	1072	525	15470	2718	907	12379	230	5261	2080	359
31	AB:B12	1A-7A	540	787	17558	703	16480	559	6354	18054	13983	5575
32	AB:F7	1A-7A	551	455	8374	7694	2452	329	996	2681	2832	941
33	AB:G7	1A-7A	632	710	8278	1018	3753	2941	624	6170	3583	1050
34	CD:C7	1A-7A	109	175	14322	3891	10183	628	76	8438	2217	976
35	CD:D8	1A-7A	824	2270	10285	4280	1681	1314	997	1715	761	710
36	CD:G4	1A-7A	177	72	29912	87	24392	103	54	18285	13673	10861
37	CD:G5	1A-7A	230	152	16874	161	6487	45	103	15209	9892	4354
38	CD:G3	1A-7A	146	178	26356	138	22005	90	96	19394	15188	10128
39	CD:G6	1A-7A	129	84	12775	115	2504	91	80	5922	3365	696
40	CD:C9	1A-7A	2293	2507	8908	3372	2634	3247	2610	3722	2296	1937
41	CD:H10	1A-7A	430	290	29772	306	23992	438	424	20508	22871	7906
42	CD:H11	1A-7A	542	227	24760	324	17835	128	131	15142	15562	10109

FIGURE 19

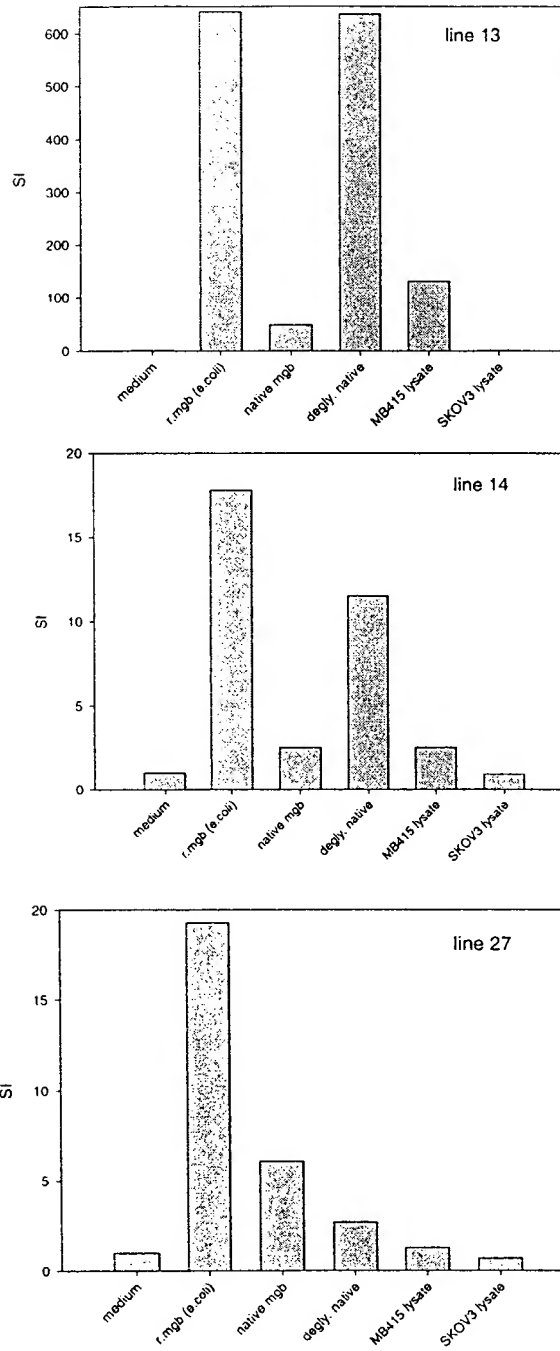


Fig. 20

H ₂ N-	Met	His tag 6aa	Ra12 (short) 30aa	HindIII 2aa	Human mammaglobin (full length) 93aa	-C00
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FIGURE 21

Ra12(s)MammFL pCRX1 Expression Screen

